SUPPLEMENTAL DATA:

Maternal Use of Electronic Cigarettes and Impact on Offspring: A Double-Hit Model

RESULTS:

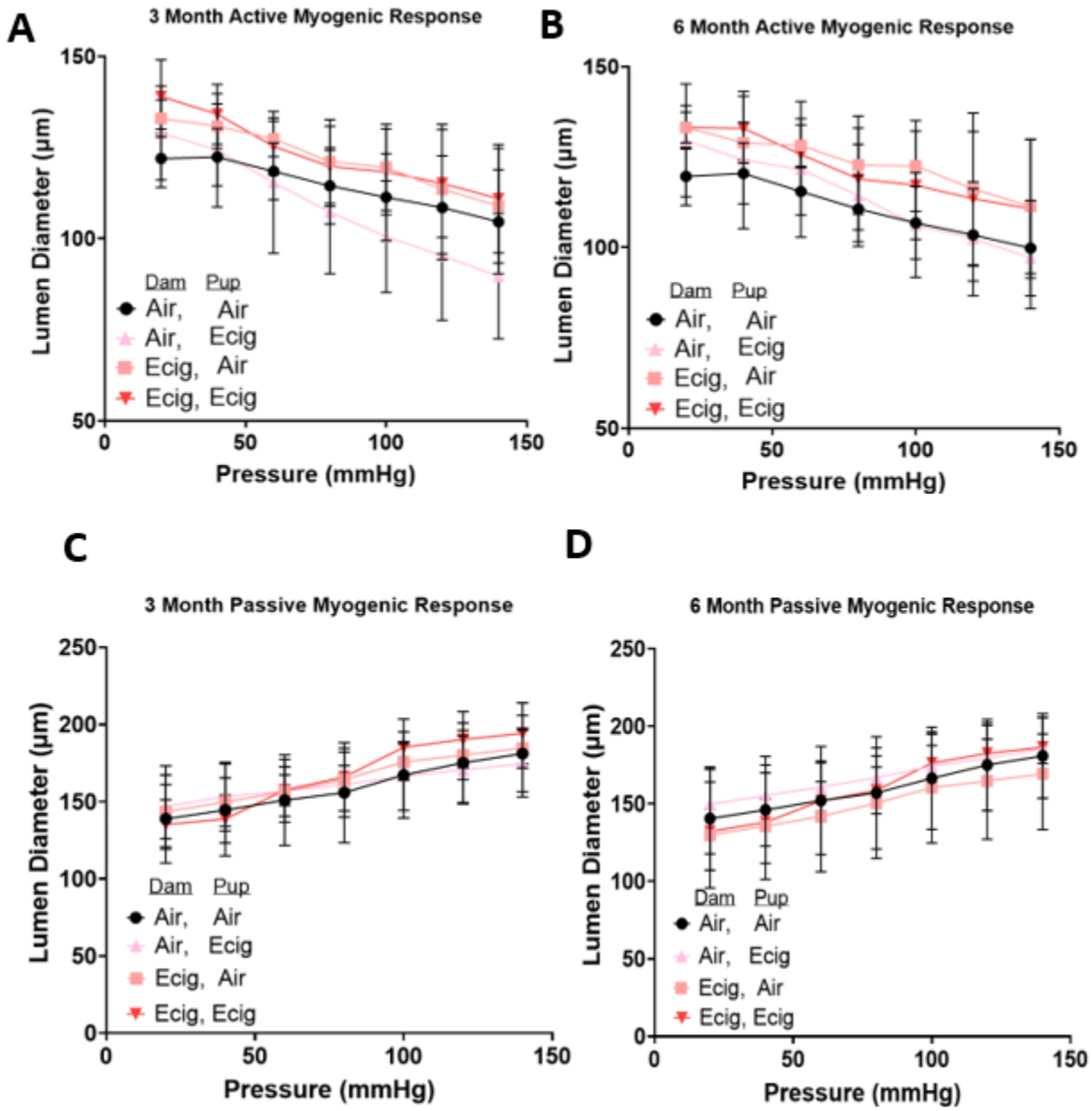
Middle Cerebral Artery Vascular Function

In addition to reactivity, the myogenic response was also assessed. Both 3- and 6-month offspring showed no differences in active myogenic response relative to Air controls (**Supplement Figure 1**). Tone, lumen diameter, wall thickness, and wall-to-lumen ratios were also measured and again there were no differences (data not shown).

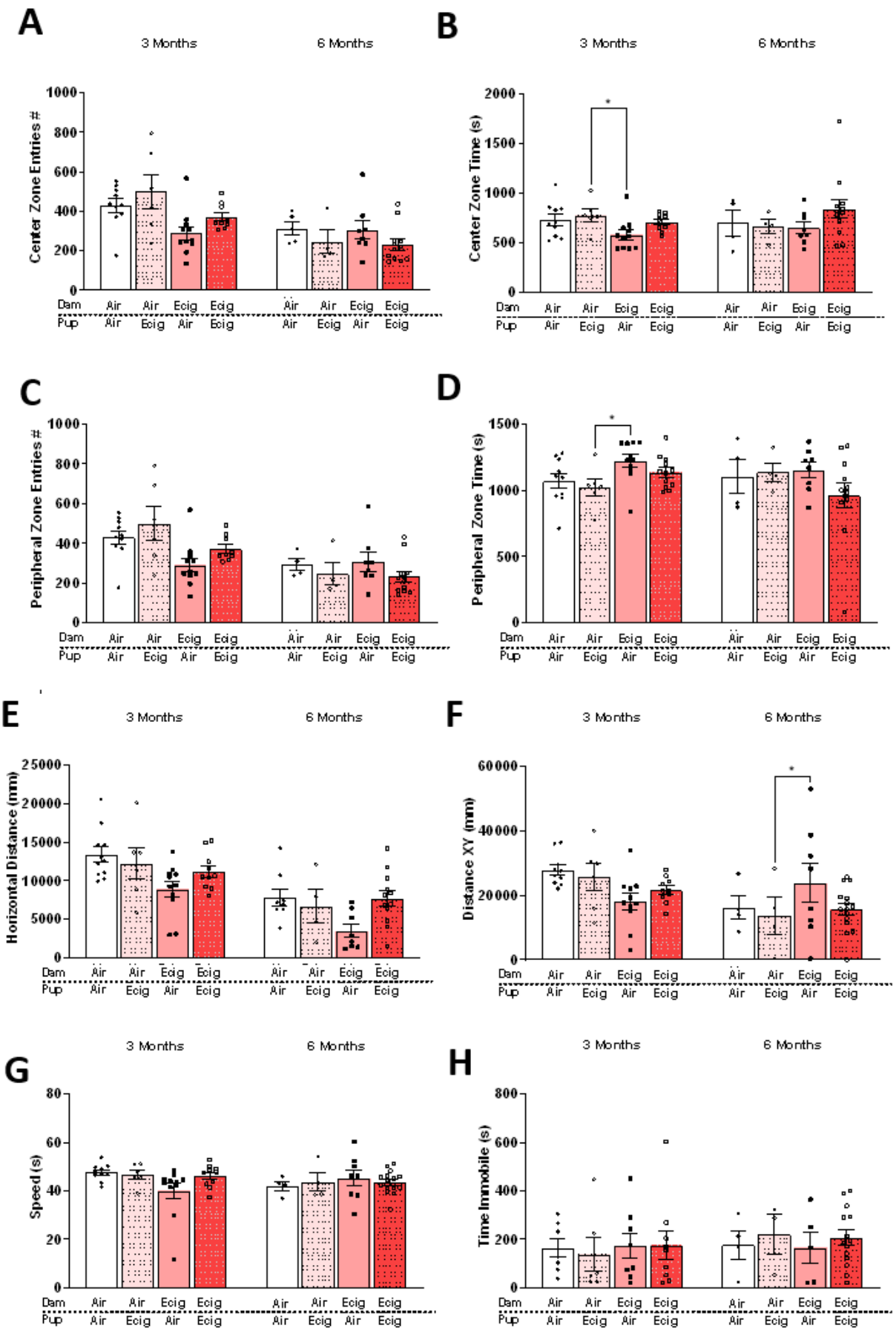
Brain Histology and Behavior Activity

Offspring locomotor activity was recorded using the open field test. At 3-months of age, all Ecig exposed groups showed a significant decrease in central tendency compared to Air controls (Figure 7A track plots and B bar graphs, $p < 0.05$). Locomotor activity was decreased in 3- and 6-month offspring compared to controls (Figure 7C, $p < 0.05$). There were no differences in rearing (Figure 7D), speed, center, and peripheral zone entries, times, horizontal distance, XY distance, and time immobile (**Supplement Figure 2**).

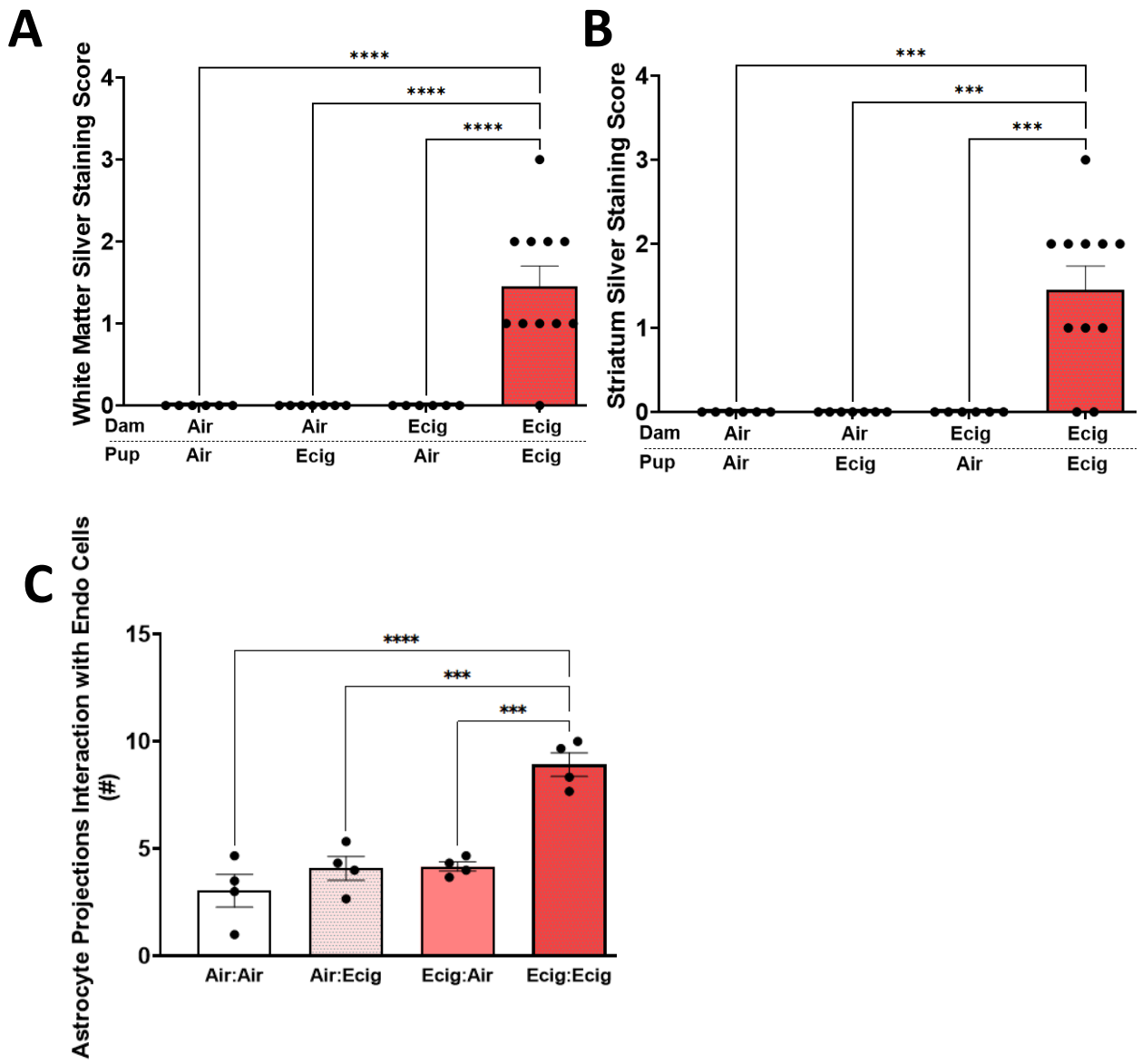
In 6-month offspring, the Ecig:Ecig exposed groups scored significantly higher in damage in the cortex and total brain score (Figure 8 B&C, $p < 0.05$), as well as white matter, and striatum (**Supplement Figure 3 A&B**). Astrocyte number was increased in all Ecig groups compared to Air controls, but only the Ecig:Ecig group showed an increase in the number of interactions between Astrocytes and Endothelial Cells (Figure 8E&F, $p < 0.05$). Astrocyte projections were also measured in proximity to endothelial cells and again the Ecig:Ecig group had more interactions compared to all other groups (**Supplement Figure 3C**).



Supplement Figure 1: Active (A, B) and Passive myogenic (C, D) response in 3- and 6-month offspring following MCA reactivity measurements.



Supplement Figure 2: Anxiety-like behavior, and locomotor activity in offspring with the Open Field test.



Supplement Figure 3: Silver staining in the White Matter and Striatum, and astrocyte projection interactions with endothelial cells.

Supplement Table 1: Blood Pressure

Dam:Offspring	**Air:Air**	**Air:Ecig**	**Ecig:Air**	**Ecig:Ecig**
Dam n=	6	6	5	5
3-month-old Offspring				
	Male			
Systolic (mmHg)	127 ± 17	121 ± 14	117 ± 17	143 ± 8.3
Diastolic (mmHg)	100 ± 13	83 ± 15	89 ± 16	110 ±15
MAP (mmHg)	109 ± 15	95 ± 14	98 ± 16	121 ± 13
Heart Rate (BPM)	391 ± 59	386 ± 27	312 ± 75	384 ± 60
	Female			
Systolic (mmHg)	129 ± 12	129 ± 22	125 ± 5.7	118 ± 21
Diastolic (mmHg)	100 ± 14	90 ± 20	99 ± 4.8	84 ± 24
MAP (mmHg)	109 ± 13	103 ± 20	108 ± 5.2	95 ± 23
Heart Rate (BPM)	417 ± 29	366 ± 24	387 ± 76	381 ± 93